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(54) Title: PEPTIDE LIBRARY AND SCREENING METHOD

(57) Abstract

A random peptide library constructed by transforming host cells with a collection of recombinant vectors that encode a fusion protein comprised of a DNA binding protein and a random peptide and also contain a binding site for the DNA binding protein can be used to screen for novel ligands. The screening method results in the formation of a complex comprising the fusion protein bound to a receptor through the random peptide ligand and to the recombinant DNA vector through the DNA binding protein.

WHAT IS CLAIMED IS:

1. A method of constructing a random peptide library of at l ast 10^6 members, said method comprising the steps of:

(a) constructing a recombinant DNA vector that encodes a DNA binding protein and contains a binding site for the DNA binding protein;

(b) inserting into the coding sequence of the DNA binding protein in at least 10^6 vectors of step (a) a coding sequence for a random peptide such that the resulting vectors encode at least 10^6 different fusion proteins, each of which is composed of the DNA binding protein and a random peptide;

(c) transforming host cells with the vectors of step (b); and

(d) culturing the host cells transformed in step (c) under conditions suitable for expression of the fusion proteins.

2. The method of Claim 1, wherein said host cell is a bacterium.

3. The method of Claim 2, wherein said bacterium is E. coli, and said recombinant DNA vector is a plasmid.

4. The method of Claim 3, wherein said DNA binding protein is selected from the group of proteins consisting of phage repressor or activator proteins, transcriptional regulators, phage 434 repressor, lambda phage cI and cro repressors, E. coli CAP protein, myc and related proteins, fos protein, jun protein, Drosophila paired protein, TFIIIA, yeast Gal4, phage P22 Arc and Mnt repressors, lac repressor, and protein complexes comprising either yeast Gal80 or adenovirus E1A protein.

5. The method of Claim 4, wherein said DNA binding protein is a lac repressor protein composed of two lac headpieces joined by a linker.

6. The method of Claim 4, wherein said DNA binding protein is the lac repressor protein, said DNA binding site is either lacO or $lacO_S$, and said plasmid contains at least two DNA binding sites.

7. The method of Claim 6, wherein said random peptide is located at the carboxy terminus of said fusion protein.

8. A method for screening a random peptide library of Claim 1, said method comprising the steps of:

(a) lysing the cells transformed with the peptide library under conditions such that the fusion protein remains bound to the vector that encodes the fusion protein;

(b) contacting the fusion proteins of the peptide library with a receptor under conditions conducive to specific peptide - receptor binding; and

(c) isolating the vector that encodes a peptide that binds to said receptor.

9. The method of Claim 8 further comprising the steps of:

(d) transforming a host cell with the vectors obtained in step (c); and repeating steps (a), (b), and (c) with the host cells transformed in step (d).

10. The method of Claim 9, wherein said host cell is E. coli.

11. The method of Claim 10, wherein said DNA binding protein is a lac repressor protein and said DNA binding site is either lacO or $lacO_S$.

12. The method of Claim 11, wherein said vector is a plasmid that contains at least two $lacO_S$ DNA binding sites.

13. A recombinant DNA vector useful for constructing a random peptide library, said vector comprising:

(a) a DNA sequence encoding a DNA binding protein;

(b) a promoter positioned so as to drive transcription of said DNA binding protein coding sequence;

(c) at least two binding sites for said DNA binding protein; and

(d) a coding sequence for a peptide inserted in said DNA binding protein coding sequence so that said coding sequences can be transcribed to produce an RNA transcript that can be translated to produce a fusion protein capable of binding to said DNA binding sites.

14. The plasmid of Claim 13, wherein said DNA binding protein is a lac repressor protein and said DNA binding sites are eith r lacO or $lacO_S$.

15. The vector of Claim 14 that is plasmid pMC3.

16. The vector of Claim 14 that is plasmid pMC5.

17. The vector of Claim 14 that is plasmid pJS123.

18. The vector of Claim 14 that is plasmid pJS141.

19. The vector of Claim 14 that is plasmid pJS142.

20. A recombinant host cell transformed with a vector of Claim 13.

21. The transformed host cell of Claim 20 that is E.coli ARI 161/pMC3.

22. The transformed host cell of Claim 20 that is E. coli ARI 161/pMC5.

23. The transformed host cell of Claim 20 that is E. coli ARI 161/pJS123.

24. The transformed host cell of Claim 20 that is E. coli ARI 246/pJS141.

25. The transformed host cell of Claim 20 that is E. coli ARI 280/pJS142.

26. A random peptide library composed of at least 10^6 different members, wherein each member is a host cell transformed with a recombinant DNA vector that encodes a DNA binding protein and contains a binding site for the DNA binding protein and a coding sequence for a random peptide inserted into the coding sequence of the DNA binding protein such that the resulting vector encodes a fusion protein that is composed of the DNA binding protein and the random peptide; and wherein each different member differs from other members with respect to the sequence of the random peptide.

27. A ligand fragment library composed of at least 10 different members, wherein each member is a host cell transformed with a recombinant DNA vector that encodes a DNA binding protein and contains a binding site for the DNA binding protein and a coding sequence for a ligand fragment inserted into the coding sequence of the DNA binding protein such that the resulting vector encodes a fusion protein that is composed of the DNA binding protein and the ligand fragment; and wherein each different member differs from other members with respect to the sequence of the ligand fragment.

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